SPERMATOCYSTS IN AEDES AEGYPTI (LINNAEUS)[1]

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It has been known for some time that groups of germinal cells in the testes of various mosquitoes are separated by delicate, membranous, transverse lamellae into a series of chambers or compartments (Hurst, 1890; Kulagin, 1907; Cholodkowsky, 1905; and Lomen, 1914) which can be referred to as *spermatocysts*. The aims of the present study were to determine the number and character of these compartments during the life span of the Bangkok strain of the Yellow Fever mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) and to ascertain whether the spermatocysts would reflect the sexual activity of adults.

Larvae were reared in batches of 100 in 250 ml. of water in stender dishes and were well-fed. Pupae were sexed by examining the difference in their external genitalia (see Christophers, 1960) and allowed to emerge as adults in a cubic-foot screened cage, where they had continuous access to sugar water. The temperature varied from 25° to 30° C. The testes were dissected into a small drop of *Drosophila* saline (Ephrussi and Beadle, 1936) with fine needles and micro-forceps, and were examined with and without phase contrast microscopy, either without coverslipping or after slight flattening under a coverglass. Such flattening was often very useful when the testes were heavily encased in fat body, but in many cases even after flattening the fat body still completely obscured one or more portions of the testes so that accurate counts could not be made. The fat body could sometimes be removed by gently pushing the coverslip with a wet piece of filter paper which caused the testes to roll over in the wet whole mount. In a number of cases the fat body jacket was seen to be stripped away as the testes were being pulled out of the body. The number of compartments was generally counted and categorized at a magnification of 430 ×. Often two to four counts were made on the same region of a single testis and these were averaged. Frequently only one portion of a testis was sufficiently visible for accurate counts; hence, total numbers of cysts in Tables II through VI include only the complete counts. Wherever possible, the character of the cells in the different compartments was categorized as either undifferentiated (= spermatogonia, spermatocytes, very early spermatids) or as partially to fully differentiated. In some cases, however, only the number of cysts could be counted. In some other cases, the presence of maturation could be detected but accurate counts were not possible. Although it was often possible to discriminate partially- from fully-differentiated spermatocysts, this distinction could not always be made with certainty. Cells in the anteriormost portion of the testes were often very in-

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distinctly separated and the counting error is generally greater for this region than for the partially- to fully-differentiated cysts. The precise ages of pupae and adults were obtained by watching for the moment of ecdysis. These studies were made in the summers of 1960, 1961 and 1966.

Observations

Larvae were dissected daily from the first through the seventh days after hatching, independently of their particular stadium. Forty-two gonads dissected from 34 larvae were identified as testes. The number of spermatocysts ranged from three in one-day-old larvae to a maximum of 24 in a 6-day-old fourth stage larva. The mean numbers of cysts for the larval period varied from 3.8 to 22.5 (Table I). Only one out of 8 testes from five-day-old larvae showed evidence of early differentiation of the germ cells in the posteriormost compartment. The cells in this case were pyriform spermatids. In none of the larvae examined were fully differentiated spermatozoa observed. Last stage larvae had from 15 to 24 testicular compartments, with an average of about 20. During larval life, the testes increased 8 to 9 times in length and changed from small ovoid organs into long pyriform structures. Most of the testes dissected from larvae were free of fat body up to the fourth stage. In fourth stage larvae, one of the testes tended to be noticeably larger than the other in the same individual. It is estimated that the number of spermatocysts increases about five times during larval life. The maximum number of cysts is reached several days before pupation.

Table I

Daily spermatocyst counts of Aedes aegypti (L.) during the larval stage

Days after hatching	No. larvae dissected	No. testes	No. counts	Number of spermatocysts	
				Range	Mean
1	4	4	6	3–5	3.8
2	5	6	10	4–10	7.3
3	5	7	7	10–18	14.3
4	6	7	15	16–22	19.9
5	7	8	11	15–21	18.0
6	4	4	2	21–24	22.5
7*	5	8	13	9–23	16.4

* Data collected 1966; other data 1960–1961.

During the pupal stage the number of spermatocysts ranged from 9 to 29, with means fluctuating around 19 (Table II), thus indicating that the number of testicular compartments remains essentially the same as that of fourth stage larvae. Although 8 out of 9 testes dissected from 6 newly emerged "white" pupae (0–8 minutes old) had from one to four cysts (Table II) with spermatids in the spindle form or with flagellum formation beginning, some other batches of newly emerged pupae (0–10 minutes old) which were examined during these studies had no differentiating cysts at all (11 testes from 6 pupae; data not shown). Indeed, a few testes had not begun to differentiate sperm for as long as 21 hours after pupation.

In some batches of pupae, a few spermatocysts were matured as early as five hours; in others, fully matured spermatozoa were not seen until near the end of the first day. The number of differentiated cysts definitely increases in old "black" pupae about to emerge as adults (that is, within pharate adults), when as many as 13 matured cysts were found in one case. The matured sperm were not active within these cysts. Sperm were never observed in the sperm ducts during the pupal stage.

Spermateleosis (process of differentiation of spermatozoa from spermatids) always begins within the posteriormost compartment of the testes and proceeds anteriorly until as much as 75% to 80% of the gonads may have differentiated cysts.

Table II

Spermatocyst counts of Aedes aegypti (L.) during the pupal stage

Age	No. dissected	No. testes	Number of cysts/testis							
			Extent of differentiation						Total†	
			None		Begun		Complete			
			Range	Mean	Range	Mean	Range	Mean	Range	Mean
0–8 min.*	6	9	11–17	14.8	0–4	2.3	0	—	13–21	17.1
25–45 min.*	5	9	14–19	16.7	1–2	1.2	0	—	15–20	17.5
3½–4 hr.	5	6	15–29	20.0	0–4	0.6	0	—	15–29	20.6
5 hr.	6	12	9–22	15.3	0–		0–5	1.8	9–23	17.0
5–6 hr.	5	8	15–24	21.3	0–		0–		15–24	21.3
16½–17½ hr.	9	14	11–23	17.7	0.4	1.5	0–		13–23	19.2
18–21 hr.	10	13	14–23	18.9	1–4	2.0	0–		15–25	19.9
21–22 hr.*	4	7	10–19	14.8	0–1	—	3–6	4.7	15–23	20.1
24 hr.	3	5	13–20	16.0	1–4#	2.2	0–8¢	3.4	18–24	21.6
27–29 hr.	3	4	11–15	13.0	—	—	4–6¢	5.0	16–20	18.0
Old pupae^φ	7	9	9–12	10.8	0–1	0.2	6–12¢	9.3	18–24	20.6
Old pupae^φ*	5	8	6–10	7.9	—	—	6–13	8.7	12–20	16.5

† Totals do not include partial data.
* 1966 data; all other data are for 1960–1961.
φ Pharate adults.
// Based on 10 measurements.
\# Based on 3 measurements.
¢ Combined partially to fully differentiated cysts.

The anteriormost region of the testis always maintains an undifferentiated zone of cysts. Although there is a tendency for a number of cysts in both testes within an individual to begin to mature at the same time, individuals have been seen where maturation had begun in one testis and not in the other. There was no correlation between the presence or absence of a lumen in the vasa efferentia and the maturation of the testes.

Within the matured testis the non-differentiated anterior portion is made up of spermatocysts in bands or layers one to three or more cells thick in optical section. The cells are relatively large and spherical and each has a large nucleus. (Before the testis matures, all of the germ cells have this appearance and the cysts are small anteriorly and progressively tend to enlarge posteriorly.) The spermatids pass through 8 stages to become mature spermatozoa (Krafsur, 1964). After passing through the pyriform and spindle stages, the cells progressively elongate and a flagellum is formed. Cysts with early differentiating spermatids do not have a distinct color. As differentiation proceeds, the cysts take on a distinct yellowish brown cast with transmitted light. Differentiating cysts tend to be larger than non-differentiated ones. The terminal cyst is the largest testicular compartment; and, after the first day of adult life, the spermatozoa actively move about within it, often

TABLE III

Spermatocyst counts on testes of unmated Aedes aegypti (L.) during the adult stage

Age and status	No. dissected	No. testes	Numbers of cysts/testis						
			Extent of differentiation						
			None		Partially to fully differentiated			Total†	
			Range	Mean	Range	Mean	%	Range	Mean
Newly emerged; unrotated	15	18	10–17	12.7	5–18	9.8	43.1	18–34	22.5
0 hour	7	12	5–11	9.2	3–14	9.5	48.2	13–25	19.7
5 hour*	6	11	5–9	7.5	7–12	9.8	56.6	15–21	17.3
¼ rotated*	5	7	7–16	10.5	8–11	9.5	48.8	16–25	20.0
10 hour (½ r)*	4	8	6–11	8.1	7–13	10.0	53.8	15–22	18.6
½ rotated	9	13	6–17	11.2	3–14	10.5	47.0	14–31	21.7
15 hour*	6	12	6–12	9.1	5–13	8.4	46.9	12–24	17.9
0–1 day	4	7	5–11	7.2	7–10	8.2	53.7	12–18	15.4
16½-24 hour; rotated	9	14	9–15	11.2	6–14	9.3	45.5	18–26	20.5
1 day	10	13	6–10	9.1	4–9	6.7	43.3	11–19	15.8
24 hour*	5	8	6–16	9.5	6–11	7.7	44.0	12–24	17.5
2 days*	11	17	4–12	6.6	3–9	5.6	45.7	7–18	11.9
3 days*	7	12	3–10	7.0	3–6	4.8	41.9	7–16	11.9
4 days*	6	11	4–9	5.5	3–6	4.4	43.6	7–15	9.9
5 days*	5	10	4–13	7.4	2–8	4.5	37.7	7–18	12.1
6 days*	5	9	5–10	6.4	2–5	3.7	34.5	7–14	10.0
7 days*	6	10	5–14	7.5	2–6	3.9	35.8	8–18	11.2
7 days	10	18	3–11	8.2	2–5	3.0	27.1	7–14	11.2
10 days*	7	13	5–10	8.0	2–6	3.4	29.8	9–16	11.4
2 weeks	6	10	4–9	6.5	2–6	3.9	35.1	8–12	10.4
2 weeks	10	20	5–16	7.4	2–6	3.8	35.4	8–13	11.2
3 weeks	10	18	3–18	5.4	2–5	2.6	32.4	5–12	8.0
4 weeks	10	18	3–9	7.0	0–5	2.7	30.3	5–12	9.7
5 weeks	5	8	3–8	4.9	2–5	2.9	36.6	5–11	7.8
6 weeks	5	8	2–8	5.3	1–5	2.2	31.5	5–13	7.5

† Totals do not include partial data.
* Data collected 1966; all other data collected 1960–1961.

in dense, violently spinning whorls. The long threadlike cells in fully differentiated compartments are tightly wound into ovoid balls, when the sperm are inactive *in situ*. The testes of pupae and especially of the adults exhibit a wide number of variations of the pyriform shape. The anterior end generally tends to be recurved and the posterior end is either cuplike or in the shape of a funnel. The testis may be bent into a C-shape. The middle portion may be compressed like a waist.

Testes were removed from unmated adults from the time of their emergence through the sixth week. The number of spermatocysts did not increase significantly at the time of adult emergence or thereafter. In the adult, the spermatocyst lamellae frequently did not prevent active sperm from being able to move from chamber to chamber in the differentiated region, but the sperm never moved into the non-differentiated zone. The number of cysts was found to range from five to 34, with means of 7.5 to 22.5 for the period of study (Table III). Undifferentiated compartments ranged from two to 18 (means of 5.3 to 12.7) and partially to fully matured cysts varied from none [2] to 18 (means of 2.2 to 10.5) (Table III).

Out of 50 individual comparisons, 8% of the adults examined had both testes of essentially the same size, while the remainder had one testis distinctly smaller (by a factor of 1.3-fold) than the other in the same individual. Both large and small testes tended to have approximately the same number and character of spermatocysts. Over the 6-week period of study, no significant change could be detected in the length of the testes in the 44 cases available for comparison. The length of the large testes ranged from 340 to 737 microns, and the small testes varied from 150 to 660 microns. There was no correlation between the size of a testis and the direction of rotation of the terminalia among 10 individuals studied in this regard.

During the first 24 hours of adult life, the male's terminalium rotates 180° and the posteriormost compartment of each testis opens and a certain number of sperm descend the spermatic duct (vas efferens plus deferens) and begin to fill the seminal vesicles. Among many recently emerged adults, sperm were not present in the vas efferens of one testis but were present in the duct of the other and subsequently generally one duct contained more sperm than the other in the same individual. In 12 individual comparisons, there was no correlation between the presence or number of sperm in the ducts and the size of the testes. While one testis clearly may be the first to provide a portion of the initial supply of sperm to the seminal vesicles, sperm from both testes are required to fill the vesicles.

During the first 24 hours of adult life, the mean number of non-differentiated cysts per testis ranged from 7.2 to 12.7 (with an overall mean of 9.6) and the differentiated cysts from 6.7 to 10.5 (with an overall mean of 9) (Table III). Although sperm begin to fill the postgonadal system during this period, no significant differences in the number or character of the cysts could be detected. With the present material a deletion of two mature cysts per testis could *not* have been detected.

Between the second and tenth days of adult life, when the sperm have already filled the postgonadal system, the mean number of undifferentiated cysts per testis ranged from 5.5 to 8.2 (with an overall mean of 7.1) and the matured cysts from 3 to 5.6 (with an overall mean of 4.2). These overall means differ from those of

[2] The duct from this testis possessed numerous spermatozoa throughout its length.

0- to one-day-old adults by 2.5 fewer undifferentiated cysts and by 4.8 fewer differentiated cysts per testis.

Jones and Wheeler (1965) reported 700 sperm in mature cysts, 740 sperm in the spermatic ducts, and from 3700 to 6309 sperm in the seminal vesicles of unmated *Aedes aegypti.* The filled postgonadal system would thus have from 4440 to 7049 spermatozoa. If these values are correct, spermatozoa within 6.3 to 10 matured cysts would be needed to fill the postgonadal system. The mean deletion of 4.8 matured cysts per testis thus fits in with this requirement, and could account for a supply of 6720 spermatozoa within the postgonadal system of *A. aegypti.* At least 2000 sperm could reach the postgonadal system during the first 24 hours after emergence, and the remainder be delivered shortly thereafter.

Table IV

Spermatocyst counts on testes of Aedes aegypti (L.) kept with approximately equal numbers of females for four to 7 weeks

Age	No. dissected	No. testes	Numbers of cysts/testis						
			Extent of differentiation					Total†	
			None		Partially to fully differentiated				
			Range	Mean	Range	Mean	%	Range	Mean
4 wks.	6	8	5–12	7.6	1–3	2.3	24.9	7–15	9.9
6 wks.	4	8	2–9	6.0	1–4	2.3	29.7	5–12	8.6
7 wks.	5	9	2–8	5.5	1–5	2.4	33.0	4–12	7.4

† Totals do not include partial data.

During the six-week period of study, the total number of spermatocysts in unmated males gradually declined from 22.5 to 7.5 (by a factor of 3); the undifferentiated cysts decreased from 12.7 to 4.9 (by a factor of 2.6); and the differentiated cysts were reduced from 10.5 to 2.2 (by a factor of 4.8) (Table III). The mean percentage of compartments with differentiated sperm decreased from 56.6% to a minimum of 27.1%, with an overall mean of 41.1% differentiated cysts for the entire 6-week period of study. Viewed as a whole, there is a distinct and significant trend for both undifferentiated and differentiated cysts to decline with age in unmated adults, that is, in the absence of any loss of sperm from the reproductive system.

If each mature cyst produces 700 spermatozoa, then the following calculations can be made from the data in Table III: (1) About 13,500 sperm are present in both testes before any or very few of them descend to the postgonadal system in the newly emerged adult. (2) After 24 hours, 2720 to 4120 sperm have left the testes. (3) During the next 9 days, there are from 4200 to 7840 sperm in the testes and from 5660 to 9300 in the postgonadal system. (4) If the number of sperm in each mature cyst does not change with time, then the amount of sperm in the testes should gradually decrease as the supply in the postgonadal system increases in unmated males. Between the second and tenth days, 3640 sperm should leave the testes, and, between the second and sixth weeks, 2380 sperm should leave the testes.

It is possible, however, that once the postgonadal system is filled, relatively few or no additional sperm would be added thereafter. If this were the case, spermatocyst walls could break down, leaving the same numbers of sperm within the differentiated region of the testes but with fewer spermatocysts detectable therein. With the present data, it is not possible to decide which of these is the case.

As shown in Table IV, data from males which had been caged continuously with approximately equal numbers of females for four, 6 and 7 weeks did not differ in number or character of spermatocysts from the data obtained from unmated males of the same ages (Table III).

Table V

The number and character of spermatocysts in Aedes aegypti (L.) after multiple copulation

Sex ratio and cohabitation time	Number males dissected and age when dissected	No. testes	Numbers of cysts/testis						
			Extent of differentiation					Total†	
			None		Partial-complete				
			Range	Mean	Range	Mean	%	Range	Mean
1:10/3 days	10/8 days	16	6–14	8.9	2–4	3.0	26.0	9–17	11.9
controls	6/8 days	11	5–14	8.8	1–7	3.7	30.2	6–20	12.2
1:20/1 day	11/3–4 days	17	3–13	8.7	2–10	4.8	36.9	10–17	13.6
controls	9/3–5 days	16	4–17	9.3	2–7	4.2	31.6	6–18	13.3
1:20/2 days	3/7 days	6	7–10	9.0	2–7	5.5	38.0	12–17	14.8
controls	6/7 days	10	6–13	8.8	2–5	3.1	26.1	9–16	11.9
1:20/4 days	3/14 days	6	6–11	8.2	2–7	3.8	31.4	10–14	12.3
controls	6/14 days	12	4–17	8.1	2–6	3.3	30.5	8–21	11.4

† Totals do not include partial data.

Since it had been found that the reproductive systems of males kept in the presence of equal numbers of females for as long as 7 weeks could not be distinguished from those of unmated controls (Table IV), a series of cages were set up containing varying combinations of previously unmated adults: (1) one cage of 10 males with 100 previously unmated females, (2) four cages of one male with 20 females and two cages of five males with 100 females. The adults were allowed to co-habit for one to four days and the males were dissected. Frequent matings were observed but the sexual history of individual males was not determined.

As shown in Table V, when the sex ratio was 1:10 or 1:20, the number and character of the spermatocysts were basically the same as those of the unmated controls. However, in 60% of the males the spermatic ducts and seminal vesicles contained very few spermatozoa and these individuals generally had noticeably reduced accessory gland secretion, particularly when the sex ratio was 1:20. Presumably, those males with a reduced supply of sperm and accessory gland material mated with more females than those whose supplies were not strikingly reduced. Thus, depletion of sperm from the postgonadal system and of accessory gland material did not appear to affect the general character of the testes.

This finding fits with the data of Jones and Wheeler (1965) which showed that, after males had been force-mated repeatedly, the posterior chamber of their testes still had many spermatozoa (mean of 741). Together these data show that after repeated matings only the sperm in the postgonadal system are used up and that testicular sperm are not drawn down to replenish the supply as it is being removed.

To explore this problem further, three cages were set up, each with five males to 30 females; and, after co-habiting for 24 hours, the males were isolated for one, two or three days before being dissected. After one day, three out of five males had shrunken seminal vesicles with very few spermatozoa, and the ducts leading to

TABLE VI

The number and character of spermatocysts in Aedes aegypti (L.) after being isolated following multiple copulation

Days isolated and sex ratio	No. testes	Numbers of cysts/testis						
		Extent of differentiation					Total†	
		None		Partial-complete				
		Range	Mean	Range	Mean	%	Range	Mean
1 day 1:6	3	7–11	8.7	1–4	2.3	21.6	10–12	11.0
2 1:6	9	6–22	12.2	1–4	2.4	22.5	8–23	14.4
3 1:6	6	5–9	6.8	3–5	3.7	36.3	9–13	10.7
2 1:20	8	7–11	8.7	2–6	3.5	28.5	9–15	12.3
controls	7	4–17	9.1	1–6	3.5	26.8	6–18	12.2
5 1:20	13	5–11	7.4	2–6	3.9	35.7	8–16	11.2
controls	13	5–10	8.0	2–6	3.4	29.8	8–16	11.4

† Totals do not include partial data.

them had very few if any sperm; the other two males had an obviously reduced supply of vesicle sperm. Two days after isolation, three out of five males had shrunken vesicles with very few spermatozoa, but two males had replenished the sperm within the seminal vesicles. After three days, four out of five males had replenished the sperm in their vesicles, and their accessory glands were filled with secretion. Only one male still had very few sperm within his vesicles. Essentially the same results were obtained with 5 males to 100 females (5 cages; co-habitation time one, two, and four days).

As shown in Table VI, when the sex ratio was 1:6, there does not appear to be much difference between the number of differentiated cysts in testes of depleted males and those of males which have largely replenished their sperm supply. If 4440 to 7049 sperm were removed from the testes to replenish their postgonadal supply, then 6 to 10 matured cysts should have been needed and this would require more sperm than would have been present within the 4.8 matured cysts which were available in both testes. Three days after being isolated from females, however, the males, after mostly replenishing their sperm supply, had *more* mature cysts than the depleted males. Since there was an *increase* of 3.5 undifferentiated cysts per testis two days after males were isolated from females, and since there was a *decrease* of 5.4 undifferentiated cysts after the sperm supply had been replenished on the third day, it seems likely that the 4.8 matured cysts were indeed all used up and then replaced by maturation of new cysts derived from the undifferentiated zone of the testes. Thus, it can be calculated that the 4.8 already matured cysts from both testes would contribute only 3360 sperm to the postgonadal system, and that the 5.4 extra undifferentiated cysts in each testis would produce a total of 10.8 matured cysts for both testes: of these 7.4 would replace and thus account for the 3.7 matured cysts seen in each testis of the replenished male, and the other 3.4 cysts would contribute 2380 sperm to the postgonadal system to bring the total supply there to 5740.

Discussion

During the larval life of *Aedes aegypti,* the testes grow in size and the germ cells greatly increase in numbers within them apparently near or around the time of each larval ecdysis. In some old larvae (pharate pupae) spermatids may just begin to differentiate in the posteriormost compartment of the testes. The general shape of the gonads does not depend upon the presence of germ cells, as evidenced by those males with agametic testes (Jones, 1961). Although agametic testes may still possess a number of compartments, mostly or entirely at the anterior end, they generally possess far fewer than do normal testes. Agametic testes are always smaller than normal, thus showing that the growing number of germ cells leads to a general increase in size of the testes (Jones, 1961).

The present observations indicate that differentiation of spermatids may begin shortly before pupation occurs (that is, in pharate pupae) or they may not begin for 6 to 21 hours. It is of considerable interest that differentiation can begin in one testis without necessarily simultaneously beginning in the other. The entire process of differentiation of spermatids into spermatozoa within a single cyst may be completed within a five-hour period. Maturation always begins in the posteriormost cyst. As many as 6 cysts may be maturing at the same time within a testis. Matured cysts were found in all pupae after the first 24 hours. Maturation of spermatocysts is preeminently a pupal event.

In old pupae just about to emerge as adults (that is, in pharate adults) 8.7 to 9.3 matured cysts were present in each testis. It can be calculated from this that there are 6090 to 6510 spermatozoa within each testis at this time.

The present calculations indicate that while sperm begin to fill the postgonadal system shortly after the adults emerge, this process is not completed until the second day of adult life. It is estimated that about 10 matured cysts are required to fill the postgonadal system with about 7000 spermatozoa and that both testes must contribute to this supply.

In unmated males the number of spermatocysts declines in the absence of any loss of sperm from the reproductive system. It is not clear whether this involves an increase in the numbers of sperm within the postgonadal system as their numbers decrease in the testes or whether there are no changes in the numbers of spermatozoa within different portions of the reproductive system but only a breakdown of spermatocyst lamellae.

After inseminating 6 females, the male quickly uses up all or nearly all of the sperm in his postgonadal system but the numbers of sperm in the testes are not immediately affected or drawn upon. When such males are isolated from females, they replenish the sperm in their reproductive system in two to three days and the numbers of spermatocysts do not clearly reflect this change. It is suggested that replenishment cannot be achieved solely by the use of all the sperm within the already matured cysts of the testes but requires the formation and maturation of about 11 extra cysts.

Summary

1. During the larval life of *Aedes aegypti* (L.), the testes greatly increase in size and numbers of germinal cells, and the number of compartments (or spermatocysts) increases about five times, to a maximum of 24, usually several days before pupation. Although the germ cells may begin the process of differentiation of spermatids into spermatozoa within the terminal cysts of the testes just before pupation occurs, fully differentiated spermatozoa were never observed in larvae. Generally one testis is smaller than the other in fourth stage larvae, and this difference tends to persist throughout life.

2. Although a significant increase in the number of spermatocysts could not be detected during pupal life, as many as 29 cysts were found among the testes examined during this period. Spermatids may transform into fully differentiated spermatozoa within five hours. While the beginning of differentiation of the spermatids may be delayed for as long as 21 hours after pupation, differentiated sperm were always found after the first 24 hours. The number of differentiated cysts increases during pupal life and it is calculated that 12,000 to 13,000 spermatozoa are formed by both testes. Spermatozoa were never observed in the spermatic ducts during the pupal stage.

3. Although a significant increase in the number of spermatocysts could not be found during adult life, a maximum of 34 cysts were found among newly emerged adults. The number of spermatocysts definitely declines with the age of unmated adults. Spermatozoa begin to fill the postgonadal system during the day of adult emergence. It is estimated that complete filling requires two days, and that the sperm in about 10 cysts are required.

4. When the male uses up most or all of the sperm within his postgonadal system after multiple matings, two to three days are required to replenish the sperm supply. It is suggested that this must involve the formation and maturation of about 11 extra cysts, most of which are needed for replacement within the testes, the others contributing to the supply of postgonadal sperm.

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